Available online at www.sciencedirect.com
ScienceDirect
Virology 365 (2007) 285–291
VIROLOGY
www.elsevier.com/locate/yviro

Influences on hepatitis B virus replication by a naturally occurring mutation in the core gene☆

Masaya Sugiyama [a], Yasuhito Tanaka [a], Fuat Kurbanov [a], Nobuaki Nakayama [b], Satoshi Mochida [b], Masashi Mizokami [a,*]

[a] *Department of Clinical Molecular Informative Medicine, Nagoya City University Graduate School of Medical Sciences, Kawasumi, Mizuho, Nagoya 467-8601, Japan*
[b] *Division of Gastroenterology and Hepatology, Internal Medicine, Saitama Medical University, 38 Morohongo, Moroyama-cho, Iruma-gun, Saitama 350-0495, Japan*

Received 17 November 2006; returned to author for revision 10 January 2007; accepted 7 April 2007
Available online 10 May 2007

Abstract

Little is known about specific naturally occurring mutations of hepatitis B virus (HBV) and underlying mechanisms of their association with fulminant hepatitis. A HBV clone isolated from a patient with fulminant hepatitis was analyzed, and the features of the particular mutations observed around furin cleavage site in core region (A2339G/G2345A) were assessed using an *in vitro* replication model. The clone belonged to genotype B with precore stop codon mutation (G1896A). Replication efficiency of 1.24-fold HBV genome in Huh-7 cells was increased in the presence of A2339G. Further *in vitro* studies using furin inhibitor indicated that the effect of the mutation was probably associated with accumulation of the full-length core protein without cleavage by furin-like protease, suggesting that a processing of the core protein might play an important role in regulation of viral replication. In conclusion, the A2339G mutation was considered as one of the viral factors involved in high replication efficiency.

Keywords: Hepatitis B virus; Core protein; Genotype; Furin; Fulminant hepatitis; Replication

Introduction

Hepatitis B virus (HBV) remains a major human pathogen. Although new infections are preventable through vaccination, new antiviral targets are being sought for the treatment of the estimated 350 million affected individuals worldwide (Lee, 1997). While acute infection with HBV resolves in the great majority of patients, in a proportion of patients HBV can induce fulminant hepatitis or go on to become chronic hepatitis. What factors influence the fulminant or chronic outcome of acute HBV infection are not fully defined. Approximately 10% of adults and 90% of children become persistent HBV carriers after HBV infection, and 1–2 million people die annually as the consequence of infection with the virus, due to liver cirrhosis and hepatocellular carcinoma.

HBV is the prototype strain of the family *Hepadonaviridea*. The virus has an approximately 3.2 kb circular, double-stranded DNA genome with four open reading frames: core (C), polymerase (P), surface (S), and X. Eight genotypes have been detected with a sequence divergence greater than 8% in the entire HBV genome (Okamoto et al., 1988) and been designated by capital alphabet letters from A (HBV/A) to H (HBV/H) in the order of their discovery (Arauz-Ruiz et al., 2002; Norder et al., 1994; Stuyver et al., 2000). The genotypes have distinct geographical distributions and are associated with differing severities of liver disease as well as response to antiviral therapies (Chu and Lok, 2002; Kao, 2002; Miyakawa and Mizokami, 2003).

The core region encodes two gene products, core protein and HBe antigen (HBeAg), translated from two different transcripts of 3.5 and 3.6 kb, respectively; the differences of the two products were; however, only the amino acids at the N-terminus. Most of the synthetic pathway of HBeAg is now clearly established. In 2003, Messageot and colleagues reported

☆ The nucleotide sequence of HBV-DNA isolates used in this study has been deposited in the international DNA database under accession number AB302095.

* Corresponding author. Fax: +81 52 842 0021.
E-mail address: mizokami@med.nagoya-cu.ac.jp (M. Mizokami).

0042-6822/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2007.04.004

that the C-terminal extremity of HBeAg was located at position 154 and that a proprotein convertase, furin, was involved in the maturation (Messageot et al., 2003).

Furin or furin-like protease is a transmembrane proprotein convertase localized in the *trans*-Golgi network (TGN), transported to the plasma membrane and then retrieved back through the endocytic pathway (Molloy et al., 1999). The consensus sequence of the furin cleavage site is RXK/RR, which has strictly required arginine residues at both the head and tail of the cleavage site (Nakayama, 1997). The furin cleavage site of the HBeAg C-terminus was reported as ^{151}RRGR^{154} (Fig. 1) (Messageot et al., 2003). Therefore, their results strongly indicate that the secretory HBeAg ends at Arg^{154}.

Recently, a patient was referred to our hospital for developing fulminant hepatitis B. Sequence analysis of the causal HBV revealed naturally occurring A2339G and G2345A mutations in the core region, and harboring the precore stop codon G1896A mutation. The replication efficiency *in vitro* of these clones was significantly higher than other clones with the G1896A mutation, which had been constructed before (unpublished data). The novel mutations may be responsible for the higher replication efficiency since the region of the mutation sites is adjacent to the furin cleavage site. To elucidate the biological properties of naturally occurring mutations, although the synthetic and secretory pathways of the core protein are not completely understood, we investigated whether the A2339G and/or G2345A mutations influence viral replication following transient transfection into human hepatoma cell lines.

Results

Influence of A2339G on HBV replication

A serum sample was obtained from a patient with fulminant hepatitis, in whom prothrombin time decreased less than 40% of the controls with hepatic encephalopathy of grade II or more within 8 weeks after the onset of the disease. The results of sequence analysis revealed unique mutation, A2339G and G2345A, in core gene. Then, we constructed plasmids with or without mutations and examined virological characteristics (Fig. 1). Huh-7 cells were transfected in 10-cm dish with 5 μg of each plasmid and harvested 2 days posttransfection. Southern blot analysis of core-associated HBV DNA in the cell lysate demonstrated that transfection of both pBj_2339 and pBj_2339/45 showed approximately 1.4-fold increase of core-associated HBV DNA as compared to the pBj_wild construct (Fig. 2A), while the transfection of pBj_2345 construct did not reveal the different result from wild type. The difference between these plasmids resides only in the mutations present in the core gene, as shown in Fig. 1. The transfection efficiency was monitored by reporter plasmids expressing secreted alkaline phosphatase (SEAP). But the correction of the transfection efficiency was not performed because each experiment in this study showed almost equal value. For immunoblot analysis, a particular monoclonal antibody was adopted. Because the anti-core protein monoclonal antibodies (HB50) recognize SPRRR repeats in the arginine-rich domain of core protein, only the full-length core protein, which is not cleaved by furin-like protease, can be detected by the HB50 antibody. Immunoblot analysis of cell lysate revealed approximately 1.3-fold increase of the core protein in pBj_2339 and pBj_2339/45 transfection (Fig. 2A) as compared to pBj_wild and pBj_2345, indicating that the A2339G mutation could be associated with the high expression of the core protein. This was not due to larger amounts of sample of the A2339G mutants, as revealed by reprobing of the same blot with anti-α-tubulin antibodies (lower panel in Figs. 2A and B). Transfection efficiency was monitored by cotransfection with a gene encoding SEAP. These results were confirmed by at least three replicates. Additionally, similar results were also obtained when these clones were used for transfection of HepG2 cells (data not shown).

The A2339G mutation obstructed the function of a cellular proprotein convertase

Precore proprotein undergoes enzymatic maturation by a protease activated both in the post-endoplasmic reticulum compartment and at the cell surface, which is a well known

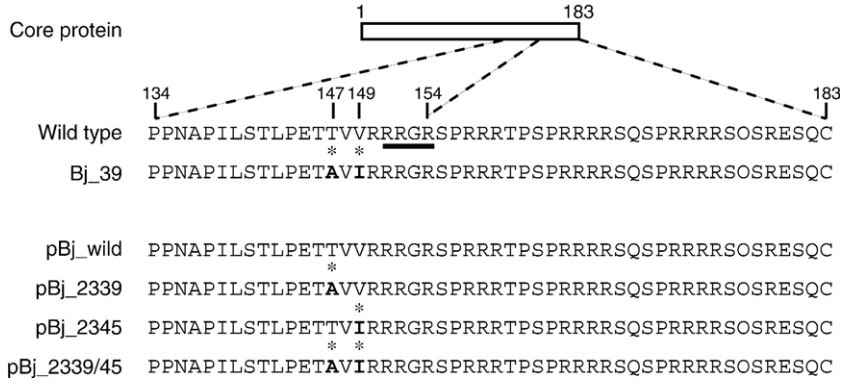

Fig. 1. C-terminus amino acid sequences and substitution mutants of the core protein. The ORF contains two in-frame initiation codons, delimiting the precore sequence and the C gene. Conventionally, position number 1 is assigned to the first amino acid of the core protein. As reported previously by Messageot *et al*., the C-terminus of the precore protein ends at Arg^{154}. The amino acid of the putative furin cleavage site is underlined. The wild-type sequence of the core protein is shown on the upper line. Names of all of the substitution mutants referred to in this paper are on the left. In each case the corresponding sequence is shown, with the mutation indicated in bold and with an asterisk.

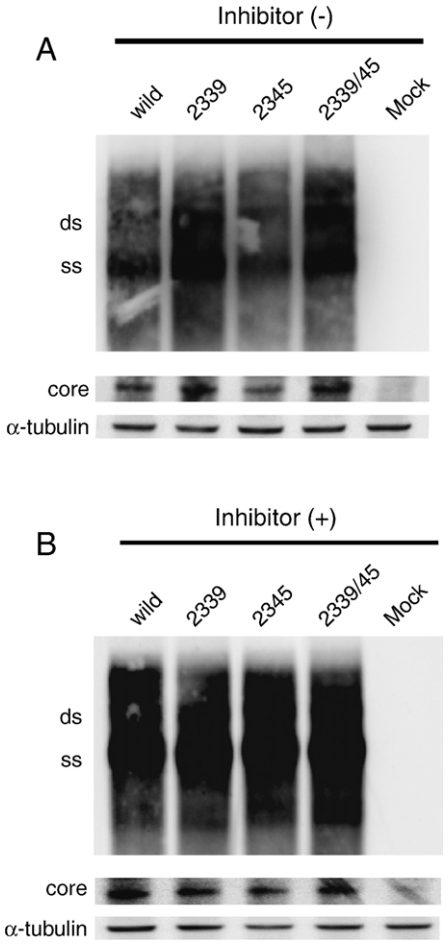

Fig. 2. Replication of the A2339G and/or G2345A mutants with or without 20 μM furin specific inhibitor, decanoyl-RVKR-chloromethylketone (dec-RVKR-cmk). Southern blot analysis of intracellular HBV replication. Isolated core-associated HBV DNAs were separated on a 1.2% agarose gel. Immunoblot analysis of core protein expression from the various constructs was performed by using HB50 monoclonal antibody. The blots were stripped of the antibodies and reprobed with a mouse anti-α-tublin antibody. (A) Without the specific inhibitor. (B) With 20 μM specific inhibitor. These results were confirmed by at least three replicates. ss: single strand DNA, ds: double strand DNA.

characteristic of furin. As the ORF of the core protein C-terminus is the same as the precore proprotein, core protein might have a similar processing step by furin. To determine whether furin-like protease is the protease involved in core maturation, a specific inhibitor, decanoyl-RVKR-chloromethylketone (dec-RVKR-cmk), was used in the next experiment. The inhibitor, at a concentration of 20 μM, was added into the medium and was maintained through to the cell lysis stage. As shown in Fig. 2B, under these conditions, the replication levels of pBj_wild and pBj_2345 became quite equal to pBj_2339 and pBj_2339/45. The expression levels of the core protein were not different between each plasmid (Fig. 2B). Thus, inhibition of the proteolytic activity of furin could induce the increase of both HBV replication and core protein expression, indicating that this protease is involved in the processing pathway of the core protein.

Influence on the replication capacities by the amount of core protein

As the replication capacity correlated well with the expression levels of core protein (Fig. 2), it would be insufficient to analyze the influence of the core protein on replication efficiency using the above replication model. To determine whether the core protein harboring the A2339G mutation avoids processing by furin-like protease, we generated expression plasmids encoding the core gene only. These plasmids drive the synthesis of wild type, A2339G, G2345A, or A2339G/G2345A core protein from the cytomegalovirus promoter, which were named pcDNA/core_wild, core_2339, core_2345, and core_2339/45, respectively. Transient expression of the core protein was achieved by transfection of Huh-7 cells with these plasmids. The transfection efficiency was monitored by SEAP. Immunoblot analysis showed higher expression levels of the uncleaved core protein for core_2339 and core_2339/45 than that for core_wild and core_2345 in conditions without furin inhibitor (Fig. 3A). In contrast, in the presence of the furin inhibitor, the amounts of full-length core protein were equal between each of the plasmid (Fig. 3B). These results were consistent with those from experiments using the 1.24-fold HBV genome as shown in Fig. 2; the A2339G mutation may enhance full-length core protein expression as well as viral replication due to the inhibition of core protein processing by furin-like protease.

No influence of furin against core protein processing

It is well-known that furin inhibitor has the ability to inhibit some proteases. To specifically silence furin, we designed

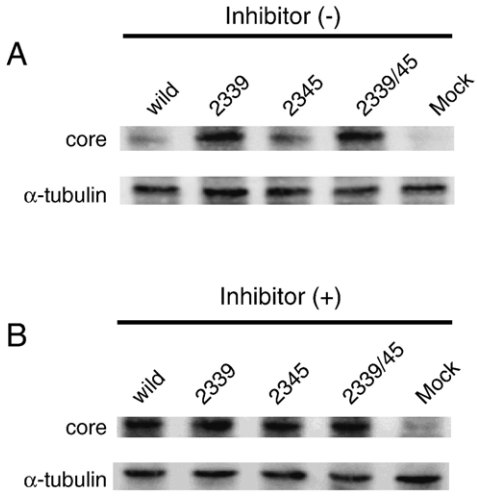

Fig. 3. Immunoblot analysis of core protein expression by the mutants expressing the core gene only. Huh-7 cells were transfected with both pcDNA/core expression vector and reporter plasmid with SEAP, and harvested 2 days posttransfection. Immunoblot analysis was performed to determine the expression levels of core protein and α-tubulin by HB50 (anti-core monoclonal antibody) and anti-α-tubulin monoclonal antibody, respectively. (A) Samples were collected from transfected cells cultured in the inhibitor-free medium. (B) Cell lysates were prepared for analysis at a concentration of 20 μM dec-RVKR-cmk.

siRNA oligonucleotides according to a previous report (Jansen et al., 2005). The ability of furin siRNA to suppress the expression of furin protein was validated by transfecting siRNA in Huh-7 cells. Immunoblot analysis revealed that the designed sequence specifically abolished furin expression in Huh-7 cells, whereas expression of α-tubulin was not affected 2 days posttransfection (Fig. 4A). As the effect of siRNA was confirmed, in addition, both HBV plasmid and siRNA were transfected 2 days after the initial transfection of siRNA. To elucidate the effect of the A2339G mutation on viral replication under down-regulation of furin, we performed southern blot analysis using purified HBV DNA from intracellular core particles additional 2 days after the second transfection. The similar trend was observed between siRNA-Control and siRNA-Furin treatment; the viral replication as well as core expression were enhanced by the A2339G mutant regardless of the furin expression levels (Fig. 4B). To confirm these results, we investigated the amount of the full-length core protein under conditions down-regulating furin expression by using a transient transfection of core expression vector derived CMV promoter. Immunoblot analysis using anti-core monoclonal antibody (HB50) showed that core expression was enhanced by the A2339G mutant regardless of the furin expression levels (Fig. 4C). Therefore, the influence of furin was not detected in these experiments.

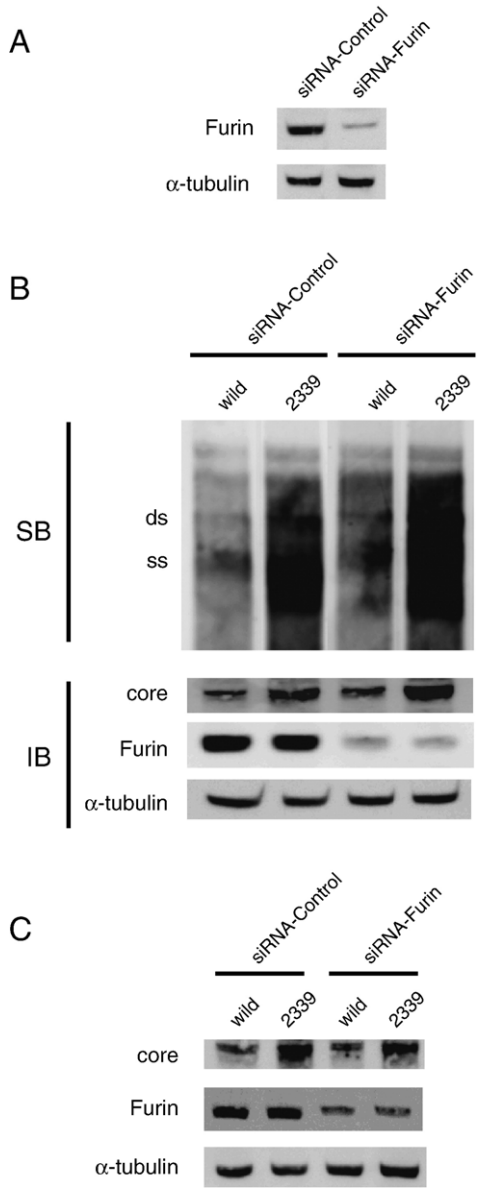

Fig. 4. Influence of viral replication and core protein expression by using siRNA specific for furin. (A) Huh-7 cells were transfected with either siRNA specific for furin or control siRNA (1 μg), and harvested 2 days posttransfection. Immunoblot analysis was performed to confirm the knock-down of furin. (B) Both siRNA (1 μg) and HBV DNA (5 μg) were transfected into cells under the knock-down of furin. The replication efficiency was analyzed by southern blot analysis and the expression levels of core protein, furin, and α-tubulin were detected by immunoblot analysis using HB50 (anti-core monoclonal antibody), anti-furin polyclonal antibody, and anti-α-tubulin monoclonal antibody, respectively. (C) The expression vector harboring core gene, either pcDNA/core_wild or pcDNA/core_2339, was transfected into Huh-7 cells 2 days after siRNA specific for furin was transfected. The levels of core expression were examined by immunoblot analysis under the knock-down of furin.

Replication of the A2339G mutant rescued by full-length core protein in trans

To elucidate whether A2339G mutant can influence the viral replication of wild-type clone *in trans*, the construct with pBj_wild or pBj_wild core(−) vector was cotransfected with either pcDNA/core_wild or pcDNA/core_2339, using a ratio 2:1. The pBj_wild core(−) construct was derived from the core_wild construct by a nonsense mutation in the core gene. As illustrated in Fig. 5, the low intra-cellular DNA level of wild-type clone was rescued by cotransfection with the core_2339 vector but not with the core_wild vector, resulting in similar replication levels of pBj_2339 and pBj_2339/45 as shown in Fig. 2. Therefore the virtue of the A2339G mutant indeed lies at the core protein levels rather than at the polymerase or the pregenomic RNA levels.

Prevalence of the A2339G mutation in GenBank

To examine the influence of the A2339G mutation on the outcomes of patients, all available published complete or partial core genome sequences of HBV were recruited from international data base (1507 entries excluding 74 from non-human primates, June 2006). Twenty-seven independent sequences with the A2339G mutation were found in the database as well as one unpublished sequence from another patient with fulminant hepatitis in our hospital. The prevalence of A2339G mutation in genotype B strains was significantly higher than that in the other genotypes (6.3% vs. 1.1%, $p<0.001$) (Table 1). Interestingly, acute exacerbation or fulminant hepatitis was found to be associated with genotype B only; 8/14 (57.1%). Six of the 8 strains had precore stop mutation (G1896A) as well as A2339G mutation.

Discussion

In the present study, the A2339G mutation was shown to enhance the replication of HBV *in vitro*. We initially investigated

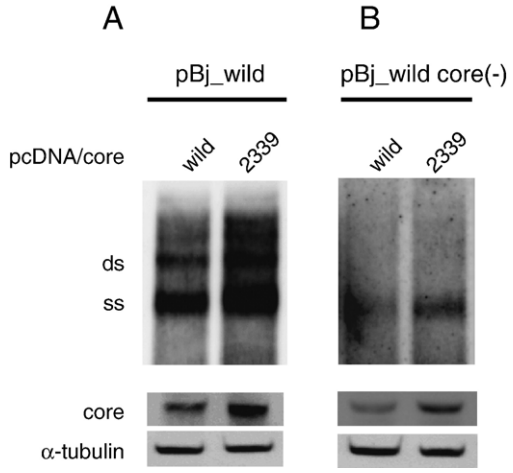

Fig. 5. Rescue of the replication of wild-type clone, either pBj_wild or pBj_wild core(−), by the core protein provided in *trans*. (A) Rescue of pBj_wild clone. (B) Rescue of pBj_wild core(−) clone. Each replication construct (5 μg) was cotransfected with 2.5 μg of pcDNA/core_wild or pcDNA/core_2339. Intracellular HBV DNA was analyzed by Southern blotting as described above. The levels of core expression in cell lysate were determined by immunoblot analysis using HB50 monoclonal antibody.

the fundamental advance effects and mechanism of the A2339G mutation against the replication of HBV, and found that acceleration of HBV replication is compatible with an increase in the level of the full-length core protein under conditions using furin inhibitor. Immunoblot analysis using core gene expression vector or the 1.24-fold HBV genome showed that the A2339G mutation may be associated with inhibition of the cleavage of the core protein by a furin-like protease, resulting in the high expression of the complete core protein. The A2339G mutation within the consensus recognition sites of furin-like protease might interfere with the recognition, binding, and/or processing by protease.

Cotransfection of the 1.24-fold the wild-type full-genome with the core protein expression vector harboring the A2339G mutation that provides core protein in *trans* enhanced the replication of HBV as compared with the wild type. Core protein with the A2339G mutation could work as a *trans*-acting regulator of HBV replication. This may reveal the reason for the aggravation of outcomes when the A2339G mutant has been shown for quasispecies or coinfections.

Furin or furin-like protease, a subtilisin-like mammalian endoprotease, is a proprotein convertase that processes latent precursor proteins into their biologically active forms. Furin is a calcium-dependent serine endoprotease that can efficiently cleave precursor proteins at paired basic amino acid processing sites (Julius et al., 1984). It is thought to be responsible for the processing of many protein precursors of viral as well as cellular origin, including gp160 and gp140 of human immunodeficiency virus type 1 (Hallenberger et al., 1992; Morikawa et al., 1993), which share the same consensus processing sites. A study to confirm and extend the concept that gp160 is processed by furin has been performed by using a cell line, LoVo, which is demonstrated to be furin-defective (Ohnishi et al., 1994). Unexpectedly, LoVo cells were shown to process gp160 as efficiently as normal cell lines do, being capable of producing fully infectious virions (although Newcastle disease virus fusion glycoprotein was not processed in the same cell lines). Thus the processing of core protein was not completely influenced by furin alone, although furin inhibitor worked in the present study. Furin inhibitor could also interfere with the activity of other proteases. These findings raise a further need to search for and identify the proteases involved in core protein processing.

HBV genotypes have distinct geographical distributions and are associated with differing severities of liver disease as well as response to antiviral therapies (Chu and Lok, 2002; Kao, 2002; Miyakawa and Mizokami, 2003). In previous studies, the majority of patients with fulminant hepatitis and fatal acute exacerbation of chronic hepatitis were found to have a G1896A precore stop codon mutation (Liang et al., 1991; Omata et al., 1991), core promoter (A1762T/G1764A) mutation, HBeAg-negative, or HBV/Bj as independent risk factors (Imamura et al., 2003; Ozasa et al., 2006). Various mutations at nt 1753 for enhanced HBV replication, as well as those adjacent at nt 1754 occurred frequently in patients with fulminant hepatitis (Imamura et al., 2003). In our study, acute exacerbation or fulminant hepatitis was relatively prevalent when outcomes of patients with the entire HBV genome or partial core sequences harboring the A2339G mutation in GenBank were analyzed. Fourteen of the sample sequences were classified into HBV/B. To our surprise, the majority (57.1%) of HBV/B sequences harboring A2339G have been reported as acute exacerbation or fulminant hepatitis while no entries with it were found in the other genotypes. Details included 4 patients with fulminant hepatitis and the remaining 4 patients with acute exacerbation of chronic hepatitis. Because of high replication levels of HBV with the A2339G mutation, patients with the mutation in the HBV genome would correlate with acute exacerbation during long-lasting HBV infection as well as in the fulminant form of acute infection.

It was reported that the A2339G mutation corresponding to core protein codon 147 was located in the epitopes of the cytotoxic T lymphocyte (CTL) in the core region (Missale et al., 1993). The CTL response to this epitope is dually restricted by

Table 1
Prevalence of A2339G mutation among HBV genotypes strains recruited from international data base

Genotype (N)	A2339G (%)	Outcomes		
		Fulminant hepatitis (with G1896A)	Acute exacerbation (with G1896A)	Chronic hepatitis (with G1896A)
A (218)	4 (1.8%)	0	0	4 (0)
B (222)	14 (6.3%)*	4 (4)	4 (2)	6 (4)
C (453)	5 (1.1%)	0	0	5 (1)
D (384)	4 (1.0%)	0	0	4 (4)
E (164)	0 (0%)	0	0	7
F (43)	0 (0%)	0	0	0
G (13)	0 (0%)	0	0	0
H (10)	1 (10%)	0	0	1 (0)
Total (1508)	28 (1.9%)	4 (4)	4 (2)	20 (9)

* $p<0.001$: Genotype B with A2339G vs. the other genotypes with it by Chi square test (Yates' correction).

the histocompatibility leukocyte antigen (HLA) A31 and HLA-Aw68 alleles. This may cause the differences in outcomes even if the mutation exists in the core region. As well, the A2339G mutation at core protein codon 147 was frequently found in 9 (29%) of 31 chronic HBV-infected children, although it has not reached a statistically significant difference between pre-HBeAg seroconversion and post-seroconversion (Ni et al., 2003). Children with the codon 147 mutation had higher peak alanine aminotransferase (ALT) levels than those without the mutant when peak ALT levels before HBeAg seroconversion were compared between these two groups.

In conclusion, by elucidating the effect of the A2339G mutation we suggested that the viral replication level might be associated with a processing of its core protein by furin-like protease. Although the mutation A2339G might be considered as one of the viral factors involved in development of fulminant hepatitis, further studies are required to investigate details to this association and its clinical implication.

Materials and methods

Patients

A serum sample was obtained from a patient with fulminant hepatitis in Saitama Medical University Hospital, in whom prothrombin time decreased less than 40% of the controls with hepatic encephalopathy of grade II or more within 8 weeks after the onset of the disease. HBV clones from the patients belong to genotype B and had precore stop codon mutation (G1896A), but not the A1762T/G1764A double mutation in the core promoter. The study design conformed to the 1975 Declaration of Helsinki, and was approved by the Ethic Committees of Institutions. An informed consent was obtained from a patient.

Plasmid constructs of HBV DNA and sequencing

A consensus clone was used for plasmid construction as reported previously (Ozasa et al., 2006). The clone was named pBj_39, which corresponds to pBj_2339/45 with A2339G and G2345A mutations in the core region. Fig. 1 illustrates the mutants assessed in this study. Each mutation was introduced into the construct by overlap extension PCR. The PCR products were digested with *Hin*dIII and *Eco*O65I for cloning back into the pBj_2339/45 construct. In addition, we generated expression plasmids encoding the core gene only. The core gene was inserted into pcDNA3.1/Hyg (Invitrogen Corp., Carlsbad, CA). These plasmids were capable of driving the synthesis of wild type, A2339G, G2345A, or A2339G/G2345A core protein from the cytomegalovirus promoter.

Cell culture and transfection

Huh-7 cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. For the standard replication assay, 10-cm-diamater dishes were seeded with 1×10^6 cells per dish. After 16 h of incubation, Huh-7 cells were transfected with 5 μg of DNA construct using the Fugene 6 transfection reagent (Roche Diagnostics, Indianapolis, IN) and harvested 48 h later. Transfection efficiency was measured by cotransfection with 0.5 μg of a reporter plasmid expressing secreted alkaline phosphatase (SEAP) and estimating SEAP enzymatic activity in the culture supernatant. Three replicate experiments were conducted for each clone. For RNAi-mediated knockdown of furin, we referred to the previous report for the sequence to design target-specific siRNA duplex against furin (Jansen et al., 2005). We obtained a 21-nt sense and antisense strand with symmetric 2-nt 3′ overhangs of identical sequences (sense siRNA: 5′-GAC CAT TCG ACC AAA CAG TdTdT-3′). Scramble oligo-ribonucleotide duplex that was not homologue to any mammalian genes was utilized as control (sense siRNA: 5′-TTC TCC GAA CGT GTC ACG TdTdT-3′) (Little et al., 2007). Transient transfections of siRNAs in Huh-7 cells were carried out using X-tremeGene siRNA Transfection Reagent (Roche Diagnostics, Indianapolis, IN).

Isolation of core-associated HBV DNA from transfected cells and Southern Blot hybridizations

HBV DNA was purified from intracellular core particles by the method described previously (Fujiwara et al., 2005). Southern blot hybridizations were performed with a full-length probe by the method described previously (Ozasa et al., 2006).

Immunoblot analysis of core protein

Huh-7 cells grown in each well of the dishes were scraped at day 2 after transfection, and the cell pellet was lysed with CellLytic-M buffer (SIGMA, St. Louis, MO). Proteins from 10 μl of lysate were separated on a 0.1% SDS–15% polyacrylamide gel and transferred to Hybond ECL nitrocellulose membrane (Amersham Biosciences Corp, Piscataway, NJ). A mouse monoclonal anti-core antibody (HB50, kindly provided by Advanced Life Science Institute Inc.) that specifically recognizes SPRRR repeats in the arginine-rich domain of the core protein was used as the primary antibodies. To control for transfection efficiency, the blots were stripped with Restore Immunoblot stripping buffer (Pierce, Rockford, IL). The blots were incubated with a mouse monoclonal α-tubulin antibody (Zymed Laboratories, South San Francisco, CA). The proteins were detected by anti-mouse antibody HRP-linked IgG (Amersham Biosciences Corp, Piscataway, NJ).

Acknowledgments

Supported in part by a grant-in-aid from the Ministry of Health, Labour and Welfare of Japan (H16-kanen-3), Uehara Memorial Foundation, and Toyoaki Foundation.

References

Arauz-Ruiz, P., Norder, H., Robertson, B.H., Magnius, L.O., 2002. Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. J. Gen. Virol. 83 (Pt 8), 2059–2073.

Chu, C.J., Lok, A.S., 2002. Clinical significance of hepatitis B virus genotypes. Hepatology 35 (5), 1274–1276.

Fujiwara, K., Tanaka, Y., Paulon, E., Orito, E., Sugiyama, M., Ito, K., Ueda, R., Mizokami, M., Naoumov, N.V., 2005. Novel type of hepatitis B virus mutation: replacement mutation involving a hepatocyte nuclear factor 1 binding site tandem repeat in chronic hepatitis B virus genotype E. J. Virol. 79 (22), 14404–14410.

Hallenberger, S., Bosch, V., Angliker, H., Shaw, E., Klenk, H.D., Garten, W., 1992. Inhibition of furin-mediated cleavage activation of HIV-1 glycoprotein gp160. Nature 360 (6402), 358–361.

Imamura, T., Yokosuka, O., Kurihara, T., Kanda, T., Fukai, K., Imazeki, F., Saisho, H., 2003. Distribution of hepatitis B viral genotypes and mutations in the core promoter and precore regions in acute forms of liver disease in patients from Chiba, Japan. Gut 52 (11), 1630–1637.

Jansen, S., Stefan, C., Creemers, J.W., Waelkens, E., Van Eynde, A., Stalmans, W., Bollen, M., 2005. Proteolytic maturation and activation of autotaxin (NPP2), a secreted metastasis-enhancing lysophospholipase D. J. Cell Sci. 118 (Pt 14), 3081–3089.

Julius, D., Schekman, R., Thorner, J., 1984. Glycosylation and processing of prepro-alpha-factor through the yeast secretory pathway. Cell 36 (2), 309–318.

Kao, J.H., 2002. Hepatitis B viral genotypes: clinical relevance and molecular characteristics. J. Gastroenterol. Hepatol. 17 (6), 643–650.

Lee, W.M., 1997. Hepatitis B virus infection. N. Engl. J. Med. 337 (24), 1733–1745.

Liang, T.J., Hasegawa, K., Rimon, N., Wands, J.R., Ben-Porath, E., 1991. A hepatitis B virus mutant associated with an epidemic of fulminant hepatitis. N. Engl. J. Med. 324 (24), 1705–1709.

Little, G.H., Bai, Y., Williams, T., Poizat, C., 2007. Nuclear calcium/calmodulin-dependent protein kinase II{delta} preferentially transmits signals to histone deacetylase 4 in cardiac cells. J. Biol. Chem. 282 (10), 7219–7231.

Messageot, F., Salhi, S., Eon, P., Rossignol, J.M., 2003. Proteolytic processing of the hepatitis B virus e antigen precursor. Cleavage at two furin consensus sequences. J. Biol. Chem. 278 (2), 891–895.

Missale, G., Redeker, A., Person, J., Fowler, P., Guilhot, S., Schlicht, H.J., Ferrari, C., Chisari, F.V., 1993. HLA-A31- and HLA-Aw68-restricted cytotoxic T cell responses to a single hepatitis B virus nucleocapsid epitope during acute viral hepatitis. J. Exp. Med. 177 (3), 751–762.

Miyakawa, Y., Mizokami, M., 2003. Classifying hepatitis B virus genotypes. Intervirology 46 (6), 329–338.

Molloy, S.S., Anderson, E.D., Jean, F., Thomas, G., 1999. Bi-cycling the furin pathway: from TGN localization to pathogen activation and embryogenesis. Trends Cell Biol. 9 (1), 28–35.

Morikawa, Y., Barsov, E., Jones, I., 1993. Legitimate and illegitimate cleavage of human immunodeficiency virus glycoproteins by furin. J. Virol. 67 (6), 3601–3604.

Nakayama, K., 1997. Furin: a mammalian subtilisin/Kex2p-like endoprotease involved in processing of a wide variety of precursor proteins. Biochem. J. 327 (Pt 3), 625–635.

Ni, Y.H., Chang, M.H., Hsu, H.Y., Tsuei, D.J., 2003. Different hepatitis B virus core gene mutations in children with chronic infection and hepatocellular carcinoma. Gut 52 (1), 122–125.

Norder, H., Courouce, A.M., Magnius, L.O., 1994. Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. Virology 198 (2), 489–503.

Ohnishi, Y., Shioda, T., Nakayama, K., Iwata, S., Gotoh, B., Hamaguchi, M., Nagai, Y., 1994. A furin-defective cell line is able to process correctly the gp160 of human immunodeficiency virus type 1. J. Virol. 68 (6), 4075–4079.

Okamoto, H., Tsuda, F., Sakugawa, H., Sastrosoewignjo, R.I., Imai, M., Miyakawa, Y., Mayumi, M., 1988. Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. J. Gen. Virol. 69 (Pt 10), 2575–2583.

Omata, M., Ehata, T., Yokosuka, O., Hosoda, K., Ohto, M., 1991. Mutations in the precore region of hepatitis B virus DNA in patients with fulminant and severe hepatitis. N. Engl. J. Med. 324 (24), 1699–1704.

Ozasa, A., Tanaka, Y., Orito, E., Sugiyama, M., Kang, J.H., Hige, S., Kuramitsu, T., Suzuki, K., Tanaka, E., Okada, S., Tokita, H., Asahina, Y., Inoue, K., Kakumu, S., Okanoue, T., Murawaki, Y., Hino, K., Onji, M., Yatsuhashi, H., Sakugawa, H., Miyakawa, Y., Ueda, R., Mizokami, M., 2006. Influence of genotypes and precore mutations on fulminant or chronic outcome of acute hepatitis B virus infection. Hepatology 44 (2), 326–334.

Stuyver, L., De Gendt, S., Van Geyt, C., Zoulim, F., Fried, M., Schinazi, R.F., Rossau, R., 2000. A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. J. Gen. Virol. 81, 67–74.